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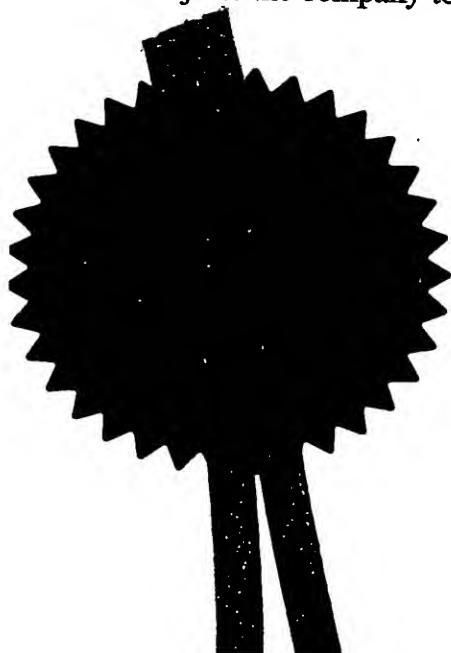
PCT

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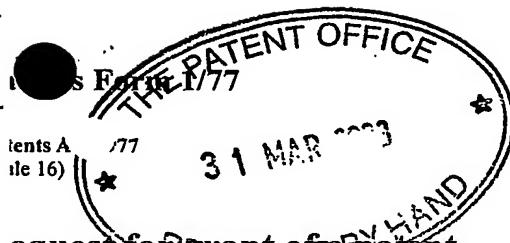
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P033703GB

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0307389.7

31 MAR 1983

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RIBOTARGETS LIMITED
Granta Park
Abington
Cambridge
CB1 6GB

7800113001

Patents ADP number (if you know it)

If the applicant is a corporate body, give the
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United Kingdom

4. Title of the invention

Amino pyrazolo[1,5-a]pyrimidine compounds and their use in medicine

5. Name of your agent (if you have one)

Carpmaels & Ransford

"Address for service" in the United Kingdom
to which all correspondence should be sent
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43 Bloomsbury Square
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WC1A 2RA

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83001

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Description	17	<i>JMH</i>
Claim(s)	3	
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Statement of inventorship and right to grant of a patent (Patents Form 7/77)

1

Request for preliminary examination and search (Patents Form 9/77)

Request for substantive examination (Patents Form 10/77)

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11.

I/We request the grant of a patent on the basis of this application.

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Carpmaels & Ransford
Carpmaels & Ransford

Date

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020-7242 8692

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Amino pyrazolo[1,5-a]pyrimidine Compounds and their Use in Medicine

This invention relates to the use of a class of substituted amino pyrazolo[1,5-a]pyrimidines in relation to diseases which are mediated by excessive or inappropriate CDK2 activity, such as cancers.

Background to the invention

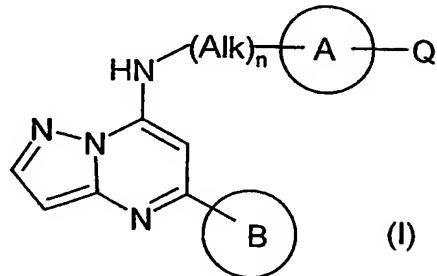
Uncontrolled cell proliferation is a hallmark of cancer. Tumor cells typically have damage to genes which play a part in regulation of the cell division cycle. Cyclin-dependent kinases (CDKs) play critical roles in regulating the transitions between different phases of the cell cycle. The serine/threonine kinase CDK2 is essential for normal cell cycling and plays a key role in disorders arising from aberrant cell cycling. Inhibitors of CDK2 are therefore useful for the treatment of various types of cancer and other conditions related to abnormal cell proliferation. Flavopyridol (M.D. Losiewicz et al., Biochem. Biophys. Res. Commun., 1994, 201, 589-595), which is currently in clinical trials, displays modest selectivity for inhibition of CDKs over other kinases but inhibits CDK1, CDK2, and CDK4 with equal potency. A purine based derivative, roscovitine (CYC-202) (W.F. De Azevedo et al., Eur. J. Biochem., 1997, 243, 518-526), similarly displays selectivity for CDKs over other kinases and is also in clinical trials.

Brief description of the invention

The present invention relates to the use of a class of amino pyrazolo[1,5-a]pyrimidine compounds as CDK2 inhibitors, for example for inhibition of cancer cell proliferation. A core 7-amino pyrazolo[1,5-a]pyrimidine ring with aromatic substitution on the amino group and in the pyrimidine ring are principle characterising features of the compounds with which the invention is concerned.

Detailed description of the invention

According to the present invention there is provided the use of a compound of formula (I) or a salt, N-oxide, hydrate or solvate thereof, in the preparation of a composition for inhibition of CDK2 activity:



wherein

Rings A and B are each optionally substituted aryl or heteroaryl,

Alk represents an optionally substituted divalent C₁-C₆ alkylene radical;

n is 0 or 1;

Q represents a radical of formula -(Alk¹)_p-(X)_r-(Alk²)_s-Z wherein in any compatible combination

Z is hydrogen or an optionally substituted carbocyclic or heterocyclic ring,

Alk¹ and Alk² are optionally substituted divalent C₁-C₆ alkylene radicals,

X represents -O-, -S-, -(C=O)-, -(C=S)-, -SO₂-, -SO-, -C(=O)O-, -OC(=O)-, -C(=O)NR^A-, -NR^AC(=O)-, -C(=S)NR^A-, -NR^AC(=S)-, -SO₂NR^A, -NR^ASO₂-, -OC(=O)NR^A-, -NR^AC(=O)O-, or -NR^A- wherein R^A is hydrogen or C₁-C₆ alkyl, and

p, r and s are independently 0 or 1.

As used herein, the term "(C_a-C_b)alkyl" wherein a and b are integers refers to a straight or branched chain alkyl radical having from a to b carbon atoms. Thus when a is 1 and b is 6, for example, the term includes methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, sec-butyl, t-butyl, n-pentyl and n-hexyl.

As used herein the term "divalent (C_a-C_b)alkylene radical" wherein a and b are integers means a saturated hydrocarbon chain having from a to b carbon atoms and two unsatisfied valences.

As used herein the term "cycloalkyl" refers to a saturated carbocyclic radical having from 3-8 carbon atoms and includes, for example, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl and cyclooctyl.

As used herein the term "aryl" refers to a mono-, bi- or tri-cyclic carbocyclic aromatic radical, and to two such radicals covalently linked to each other, Illustrative of such radicals are phenyl, biphenyl and naphthyl.

As used herein the term "carbocyclic" refers to a cyclic radical whose ring atoms are all carbon and to two such cyclic radicals covalently linked to each other, and includes aryl, and cycloalkyl radicals.

As used herein the term "heteroaryl" refers to a mono-, bi- or tri-cyclic aromatic radical containing one or more heteroatoms selected from S, N and O. Illustrative of such radicals are thienyl, benzthienyl, furyl, benzfuryl, pyrrolyl, imidazolyl, benzimidazolyl, thiazolyl, benzthiazolyl, isothiazolyl, benzisothiazolyl, pyrazolyl, oxazolyl, benzoxazolyl, isoxazolyl, benzisoxazolyl, isothiazolyl, triazolyl, benztriazolyl, thiadiazolyl, oxadiazolyl, pyridinyl, pyridazinyl, pyrimidinyl, pyrazinyl, triazinyl, indolyl and indazolyl.

As used herein the unqualified term "heterocycl" or "heterocyclic" includes "heteroaryl" as defined above, and in particular means a mono-, bi- or tri-cyclic non-aromatic radical containing one or more heteroatoms selected from S, N and O, and to groups consisting of a monocyclic non-aromatic radical containing one or more such heteroatoms which is covalently linked to

another such radical or to a monocyclic carbocyclic radical. Illustrative of such radicals are pyrrolyl, furanyl, thienyl, piperidinyl, imidazolyl, oxazolyl, isoxazolyl, thiazolyl, thiadiazolyl, pyrazolyl, pyridinyl, pyrrolidinyl, pyrimidinyl, morpholinyl, piperazinyl, indolyl, morpholinyl, benzfuranyl, pyranyl, isoxazolyl, benzimidazolyl, methylenedioxyphenyl, ethylenedioxyphenyl, maleimido and succinimido groups.

Unless otherwise specified in the context in which it occurs, the term "substituted" as applied to any moiety herein means substituted with at least one substituent selected from (C₁-C₆)alkyl, (C₁-C₆)alkoxy, hydroxy, hydroxy(C₁-C₆)alkyl, mercapto, mercapto(C₁-C₆)alkyl, (C₁-C₆)alkylthio, halo (including fluoro and chloro), trifluoromethyl, trifluoromethoxy, nitro, nitrile (-CN), oxo, phenyl, phenoxy, benzyl, benzyloxy, monocyclic carbocyclic or heterocyclic having from 5 to 7 ring atoms, -COOH, -COOR^A, -COR^A, -SO₂R^A, -CONH₂, -SO₂NH₂, -CONHR^A, -SO₂NHR^A, -CONR^AR^B, -SO₂NR^AR^B, -NH₂, -NHR^A, -NR^AR^B, -OCONH₂, -OCONHR^A, -OCONR^AR^B, -NHCOR^A, -NHSO₂R^A, -NHCOOR^A, -NR^BCOOR^A, -NHSO₂OR^A, -NR^BSO₂OR^A, -NHCONH₂, -NR^ACONH₂, -NHCONHR^B, -NR^ACONHR^B, -NHCONR^AR^B, or -NR^ACONR^AR^B wherein R^A and R^B are independently a (C₁-C₆)alkyl group. The term "optional substituent" means one of the foregoing substituent groups.

As used herein the term "salt" includes base addition, acid addition and quaternary salts. Compounds of the invention which are acidic can form salts, including pharmaceutically or veterinarily acceptable salts, with bases such as alkali metal hydroxides, e.g. sodium and potassium hydroxides; alkaline earth metal hydroxides e.g. calcium, barium and magnesium hydroxides; with organic bases e.g. N-ethyl piperidine, dibenzylamine and the like. Those compounds (I) which are basic can form salts, including pharmaceutically or veterinarily acceptable salts with inorganic acids, e.g. with hydrohalic acids such as hydrochloric or hydrobromic acids, sulphuric acid, nitric acid or phosphoric acid and the like, and with organic acids e.g. with acetic, tartaric, succinic, fumaric, maleic, malic, salicylic, citric, methanesulphonic and p-toluene sulphonic acids and the like.

Some compounds of the invention contain one or more actual or potential chiral centres because of the presence of asymmetric carbon atoms. The presence of several asymmetric carbon atoms gives rise to a number of diastereoisomers with R or S stereochemistry at each chiral centre. The invention includes all such diastereoisomers and mixtures thereof.

The rings A and B

Examples of rings A and B include phenyl, naphthyl, 2-, 3- and 4-pyridyl, 5-pyrimidinyl, 2- and 3-thienyl, 2- and 3-furyl. Currently it is preferred that rings A and B are each a phenyl ring.

Rings A and B may be optionally substituted by any of the substituents listed above in the definition of "optionally substituted". Examples of optional substituents on ring A or ring B include methyl, ethyl, methylenedioxy, ethylenedioxy, methoxy, ethoxy, methylthio, ethylthio, hydroxy, hydroxymethyl, hydroxyethyl, mercapto, mercaptomethyl, mercaptoethyl, amino, mono- and di-methylamino, mono- and di-ethylamino, fluoro, chloro, bromo, cyano, N-morpholino, N-piperidinyl, N-piperazinyl (the latter being optionally C₁-C₆ alkyl- or benzyl-substituted on the free ring nitrogen).

The radical -(Alk)_n-

When present, the Alk radical acts as a spacer radical between the amino group on the pyrazolo[1,5-a]pyrimidine ring and the ring A, and may be, for example -CH₂-, -CH₂CH₂-, -CH₂CH(CH₃)-, CH₂CH₂CH₂-, [-CH=CH-, -CH₂CH=CH-, -CH₂CH=CHCH₂-, -CH=CHCH=CH-.-C≡C-, -CH₂C≡C-, or -CH₂C≡CCH₂-]. Presently it is preferred that Alk, when present be -CH₂- or -CH₂CH₂-.

However, in another preferred class of compounds with which the invention is concerned, n may be 0 so that the ring A is directly linked to the amino group on the pyrazolo[1,5-a]pyrimidine ring.

The Q Substituent of the Ring A

In the simplest structures with which the invention is concerned, each of p, r and s may be 0, and Z may be hydrogen, so that ring A is simply aryl or heteroaryl, optionally substituted as discussed above.

In other simple structures, p, r and s may again each be 0, and Z may be an optionally substituted carbocyclic or heterocyclic ring, for example phenyl, cyclohexyl, pyridyl, morpholino, piperidinyl, or piperazyl ring. In such cases, Q is a direct substituent in the optionally substituted ring A.

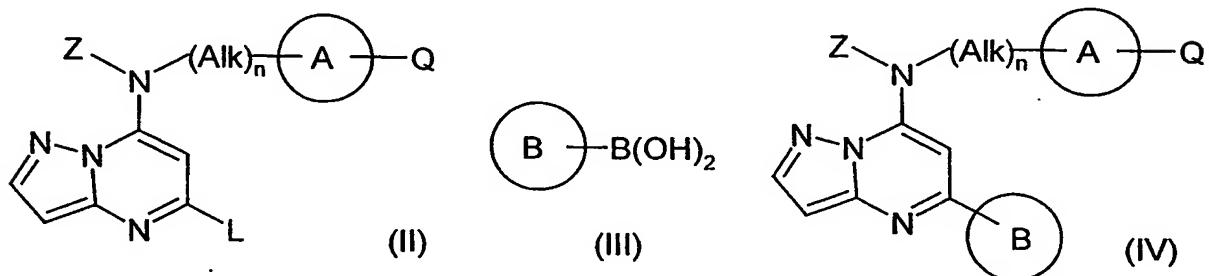
In more complex structures with which the invention is concerned, one or more of p, r and s may be 1, and Z may be hydrogen or an optionally substituted carbocyclic or heterocyclic ring. For example, p and/or s may be 1 and r may be 0, so that Z is linked to ring A by an alkylene radical, for example a C₁-C₃ alkylene radical, which is optionally substituted. In other cases each of p, r, and s may be 1, in which cases, Z is linked to Ar¹ by an alkylene radical which is interrupted by the hetero atom-containing X radical. In still other cases, p and s may be 0 and r may be 1, in which case Z is linked to Ar¹ via the hetero atom-containing X radical.

In one preferred embodiment, p is 0, r is 1, and X is a sulfonamide radical -NR^ASO₂- or a carboxamide radical -NR^AC(=O)- (R^A being as defined above, but preferably hydrogen), with the N atom linked to the ring A. In such cases s may be 1 and Z may be hydrogen, so that the group Q is an alkylsulfonamido or carboxamido substituent in the ring A; or s may be 0 and Q may be an optionally substituted carbocyclic or heterocyclic ring such as optionally substituted phenyl, eg 4-methylphenyl, so that the group Q is an optionally substituted phenylsulfonamido or carboxamido substituent in the ring A.

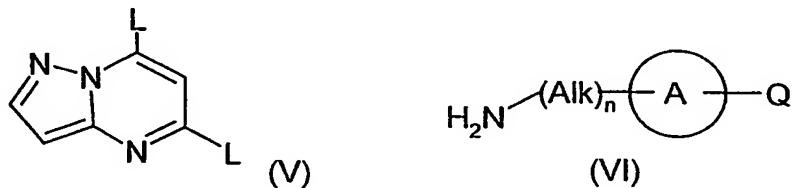
Specific compounds with which the invention is concerned include those identified in the Examples.

Compounds with which the invention is concerned may be prepared by literature methods, such as those of the preparative Examples herein, and methods analogous thereto.

For example, a compound of formula (II) wherein Z is an N-protecting group may be reacted with the corresponding aryl or heteroaryl borohydride compound (III) to prepare an intermediate compound (IV), from which the N-protecting group Z may be removed to prepare the desired compound (I).



The starting compound (II) may be prepared by reaction of a compound (VI) with an amine (V):



In the above formulae (II) – (VI), L signifies a leaving group such as halo, for example chloro. Rings A and B, Alk, Q and n are as defined in relation to formula (I).

The compounds of the invention are inhibitors of CDK2 and are thus useful in the treatment of diseases which are mediated by excessive or inappropriate CDK2 activity such as cancers, leukemias and other disease states associated with uncontrolled cell proliferation such as psoriasis and restenosis

Accordingly, the invention also provides:

- (i) a method of treatment of diseases or conditions mediated by excessive or inappropriate CDK2 activity in mammals, particularly humans, which method comprises administering to the mammal an amount of a compound of formula (I) as defined above, or a salt, hydrate or solvate thereof, effective to inhibit said CDK2 activity.; and
- (ii) a compound of formula (I) as defined above, or a salt hydrate or solvate thereof, for use in human or veterinary medicine, particularly in the treatment of diseases or conditions mediated by excessive or inappropriate CDK2 activity;

It will be understood that the specific dose level for any particular patient will depend upon a variety of factors including the activity of the specific compound employed, the age, body weight, general health, sex, diet, time of administration, route of administration, rate of excretion, drug combination and the causative mechanism and severity of the particular disease undergoing therapy. In general, a suitable dose for orally administrable formulations will usually be in the range of 0.1 to 3000 mg once, twice or three times per day, or the equivalent daily amount administered by infusion or other routes. However, optimum dose levels and frequency of dosing will be determined by clinical trials as is conventional in the art.

The compounds with which the invention is concerned may be prepared for administration by any route consistent with their pharmacokinetic properties. The orally administrable compositions may be in the form of tablets, capsules, powders, granules, lozenges, liquid or gel preparations, such as oral, topical, or sterile parenteral solutions or suspensions. Tablets and capsules for oral administration may be in unit dose presentation form, and may contain conventional excipients such as binding agents, for example syrup, acacia, gelatin, sorbitol, tragacanth, or polyvinyl-pyrrolidone; fillers for example lactose, sugar, maize-starch, calcium phosphate, sorbitol or glycine; tabletting lubricant, for example magnesium stearate, talc, polyethylene glycol or silica; disintegrants for example potato starch, or acceptable wetting agents such as sodium lauryl sulphate. The tablets may be coated according to methods well

known in normal pharmaceutical practice. Oral liquid preparations may be in the form of, for example, aqueous or oily suspensions, solutions, emulsions, syrups or elixirs, or may be presented as a dry product for reconstitution with water or other suitable vehicle before use. Such liquid preparations may contain conventional additives such as suspending agents, for example sorbitol, syrup, methyl cellulose, glucose syrup, gelatin hydrogenated edible fats; emulsifying agents, for example lecithin, sorbitan monooleate, or acacia; non-aqueous vehicles (which may include edible oils), for example almond oil, fractionated coconut oil, oily esters such as glycerine, propylene glycol, or ethyl alcohol; preservatives, for example methyl or propyl p-hydroxybenzoate or sorbic acid, and if desired conventional flavouring or colouring agents.

For topical application to the skin, the drug may be made up into a cream, lotion or ointment. Cream or ointment formulations which may be used for the drug are conventional formulations well known in the art, for example as described in standard textbooks of pharmaceutics such as the British Pharmacopoeia.

The active ingredient may also be administered parenterally in a sterile medium. Depending on the vehicle and concentration used, the drug can either be suspended or dissolved in the vehicle. Advantageously, adjuvants such as a local anaesthetic, preservative and buffering agents can be dissolved in the vehicle.

The following non-limiting Examples illustrate the the invention:

In the Examples, reactions that are specified as being carried out in a microwave oven were conducted in a Smith Synthesizer. Proton NMR experiments were conducted on a Bruker DPX400 ultra shield NMR spectrometer in the solvent specified.

HPLC: HP1100
 Column: Luna 3µm, C18(2), 30mm x 4.6mm i.d. from Phenomenex
 Temperature: 22°C
 Solvents: A - Water + 10mmol ammonium acetate + 0.08% (v/v)
 formic acid
 B – 95% Acetonitrile / 5% Solvent A + 0.08% (v/v) formic
 acid
 Flow rate: 2ml/min

Time (mins)	% Solvent A	% Solvent B	Flow (ml/min)
0	95	5	2
0.25	95	5	2
2.50	5	95	2
2.55	5	95	3
3.60	5	95	3
3.65	5	95	3
3.70	5	95	2
3.75	95	5	2

Gradient

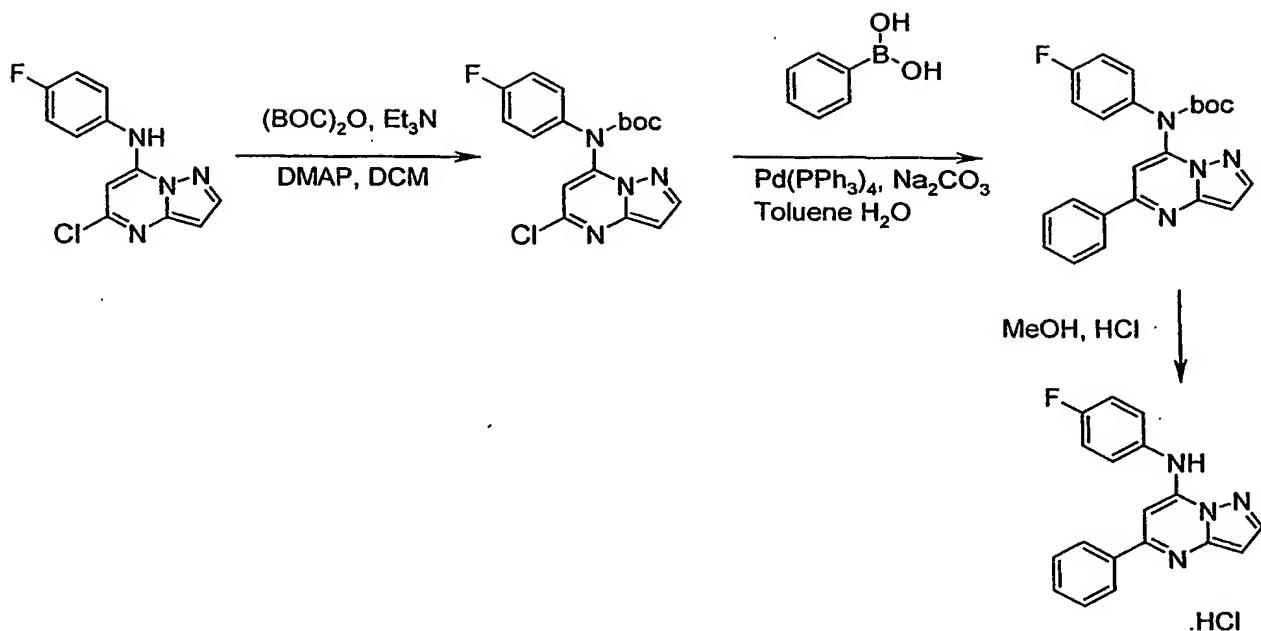
Total acquisition time is 3.75minutes

Detection: UV detection at 230nm, 254nm and 270nm

Mass Spec: HP1100 MSD, Series A

Ionisation is positive or negative ion electrospray

Molecular weight scan range is 120-1000

Example 1**Step 1****5-Chloro-7-(4-fluorophenylamino)pyrazolo[1,5-a]pyrimidine**

To a solution of 5,7-dichloropyrazolo[1,5-a]pyrimidine¹ (0.35 g, 1.86 mmol) in ethanol (15 cm³) was added 4-fluoroaniline (0.35 cm³, 3.72 mmol). The reaction mixture was heated under reflux for 1 hour. The reaction mixture was concentrated *in vacuo* and the product purified on silica eluting with 15% ethyl acetate in hexanes, to yield the title compound as a white solid (0.42 g, 86%).
 δ_H (400 MHz; d_4 -MeOH) 8.02 (1 H, d, J 2.2 Hz), 7.40-7.36 (2 H, m), 7.21 (2H, t, J 6.7), 6.32 (1 H, d, J 2.2 Hz), 5.97 (1H, s).
m/z 263 and 265 (each $M+H$, 100% and 30%) retention time 2.54 min (Method A).

Step 2**5-Chloro-7-(*N*-tert-butoxycarbonyl-4-fluorophenylamino)pyrazolo[1,5-a]pyrimidine**

To a solution of 5-chloro-7-(4-fluorophenylamino)pyrazolo[1,5-a]pyrimidine (0.15 g, 0.57 mmol) in dichloromethane (10 cm³) was added di-*tert*-butyl dicarbonate (0.37 g, 1.71 mmol), triethylamine (0.096 cm³, 0.69 mmol) and 4-dimethylaminopyridine (0.01 g, 0.082 mmol). The reaction mixture was stirred at room temperature for 16 h. The reaction was diluted with water (30 cm³) and extracted with dichloromethane (3 × 20 cm³). The combined organic fractions were washed with brine then dried with magnesium sulphate and concentrated *in vacuo*. The product was purified on silica eluting with 20% ethyl acetate in hexanes, to yield the title compound as a white solid (0.191 g, 92%).

δ_H (400 MHz; d-CHCl₃) 8.09 (1 H, d, *J* 2.3 Hz), 7.29-7.25 (2 H, m), 6.99 (2H, t, *J* 8.1), 6.63 (1 H, d, *J* 2.3 Hz), 6.60 (1H, s), 1.30 (9H, s).

Step 3

5-Phenyl-7-(*N*-*tert*-butoxycarbonyl-4-fluorophenylamino)pyrazolo[1,5-a]pyrimidine

To a solution of 5-chloro-7-(*N*-*tert*-butoxycarbonyl-4-fluorophenylamino)pyrazolo[1,5-a]pyrimidine (0.05 g, 0.14 mmol) in toluene (3.5 cm³) and water (1 cm³) was added phenyl boronic acid (0.02 g, 0.16 mmol) and sodium carbonate (0.031 g, 0.29 mmol). The solution was degassed by bubbling nitrogen through the reaction mixture for 5 min. Tetrakis(triphenylphosphine)palladium(0) (0.015 g, 0.012 mmol) was added to the mixture and the reaction was heated at reflux for 16 h. The reaction mixture was concentrated *in vacuo* and purified on silica eluting with 20% ethyl acetate in hexanes to yield the title compound as an off-white solid (0.048 g, 86%).

δ_H (400 MHz; d-CHCl₃) 8.11 (1 H, d, *J* 2.3 Hz), 7.99-7.97 (2 H, m), 7.44-7.42 (3H, m), 7.34-7.31 (2 H, m), 7.08 (1H, s), 6.97 (2H, t, *J* 8.3 Hz), 6.73 (1H, d, *J* 2.3 Hz), 1.31 (9H, s).

m/z 405 (M+H, 80%), 349 (M+H-56, 70%), 305 (M+H-100, 100%), retention time 2.92 min (Method A).

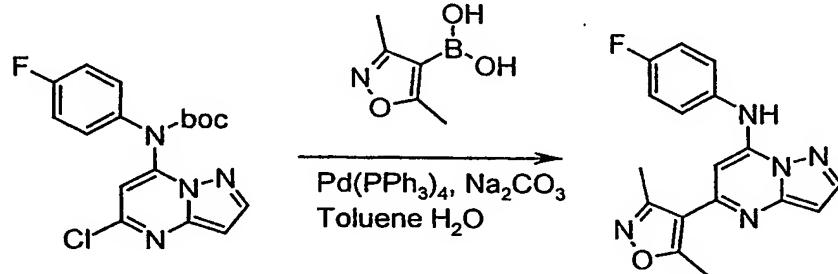
Step 4**5-Phenyl-7-(4-fluorophenylamino)pyrazolo[1,5-a]pyrimidine hydrochloride**

To a solution of 5-phenyl-7-(*N*-tert-butoxycarbonyl-4-fluorophenylamino)pyrazolo[1,5-a]pyrimidine (0.045 g, 0.11 mmol) in methanol (1 cm³) was added a solution of hydrochloric acid (3 M in methanol, 10 cm³).

The reaction mixture was stirred at room temperature for 3 h then concentrated *in vacuo*. The product was purified by crystallisation from ethyl acetate, to yield the title compound as a white solid (0.016 g, 42%).

δ_H (400 MHz; d₄-MeOH) 8.25 (1 H, d, J 2.2 Hz), 7.74-7.72 (2 H, m), 7.59-7.51 (5H, m), 7.27 (2H, t, J 8.6 Hz), 6.60 (1H, d, J 2.2 Hz), 6.39 (1H, s).

m/z 305 (M+H, 100%), retention time 2.68 min (Method A).

Example 2**5-(3,5-Dimethylisoxazole)-7-(4-fluorophenylamino)pyrazolo[1,5-a]pyrimidine**

To a solution of 5-chloro-7-(*N*-tert-butoxycarbonyl-4-fluorophenylamino)pyrazolo[1,5-a]pyrimidine (Example 1, Step 2) (0.05 g, 0.14 mmol) in 1,4-dioxane (3.5 cm³) and water (1 cm³) was added 3,5-dimethylisoxazole-4-boronic acid (0.023 g, 0.16 mmol) and sodium carbonate (0.031 g, 0.29 mmol). The solution was degassed by bubbling nitrogen through the mixture for 5 min. Tetrakis(triphenylphosphine)palladium(0) (0.015 g, 0.012 mmol) was added to the mixture and the reaction heated at 150°C for

10 min in a microwave oven. The reaction mixture was concentrated *in vacuo* and purified on silica eluting with 2% methanol in dichloromethane to yield the title compound as a white solid (0.021 g, 47%).

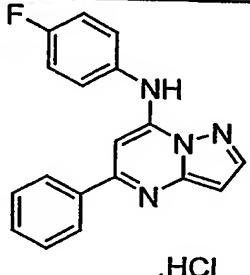
δ_H (400 MHz; d-CHCl₃) 8.03 (1 H, d, *J* 2.3 Hz), 7.97 (1 H, s), 7.33-7.30 (2H, m), 7.13 (2H, t, *J* 8.5 Hz), 6.52 (1H, d, *J* 2.3 Hz), 6.13 (1H, s), 2.50 (3H, s), 2.34 (3H, s).

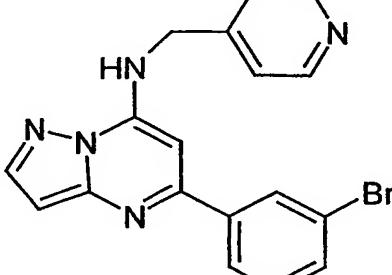
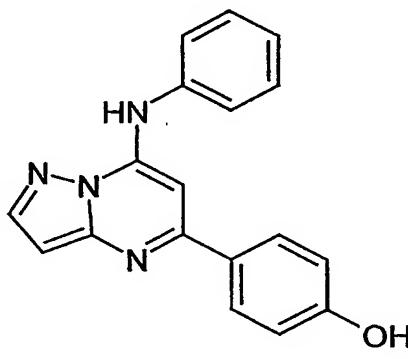
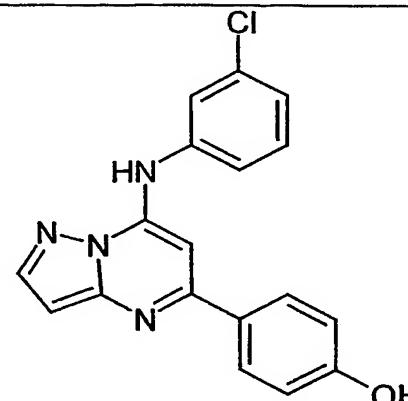
m/z 324 (*M*+H, 100%), retention time 2.51 min (Method A).

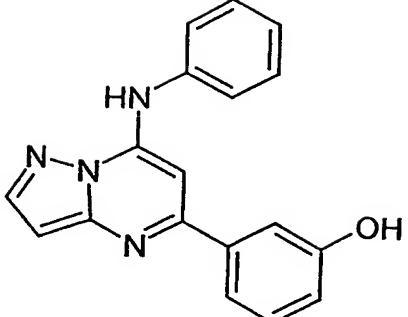
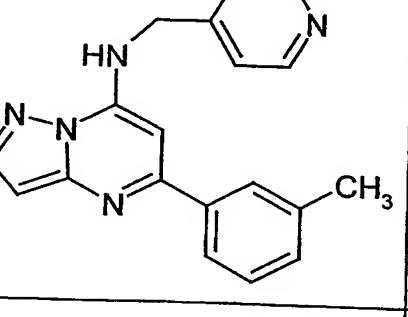
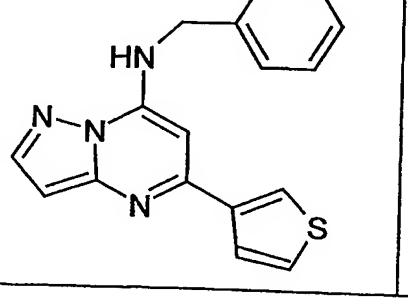
1. T. Novinson et al., Journal of Medicinal Chemistry (1976), 19(4), 512-16.

Examples 3 - 8

The compounds of Examples 3 – 8, listed in the following Table were commercially available from BioFocus (BioFocus plc, Chesterford Park, Saffron Walden, Essex, CB10 1XL). The compounds of Examples 1 and 2 are also included in the Table. All compounds were tested for CDK2 inhibitory activity in the assay described below in the Assay section. The result obtained in each case is given in the Table.

Structure	Example No	CDK2 IC ₅₀ (nM)
 .HCl	1	5.5

	2	13
	3	2.0
	4	3.8
	5	4.0

	6	5.7
	7	7.2
	8	7.6

Assay Conditions:

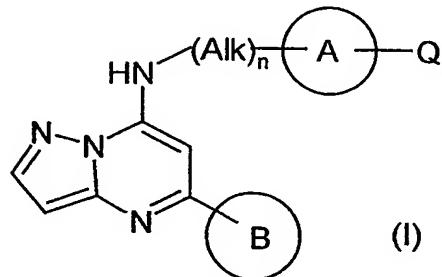
Assays for the cyclin dependent kinase activity were carried out by monitoring the phosphorylation of a synthetic peptide, HATTPKKKRK. The assay mixture containing the inhibitor and CDK-2 enzyme, complexed with cyclin A (0.4U/ml) was mixed together in a microtiter plate in a final volume of 50 μ l and incubated for 40 min at 30°C. The assay mixture contained 0.1 mM unlabeled ATP, 0.01 μ Ci/ μ l 33 P- γ -ATP, 0.03mM peptide, 0.1mg/ml BSA, 7.5mM magnesium acetate, 50mM HEPES-NaOH, pH 7.5. The reaction was stopped by adding 50 μ l of 50mM phosphoric acid. 90 μ l of the mixture were transferred to a pre-wetted 96-well Multiscreen MAPHNOB filtration plate (Millipore) and filtered on a vacuum manifold. The filter plate was washed with 3 successive additions of 200 μ l 50mM phosphoric acid and then with 100 μ l methanol. The

filtration plate was dried for 10 min at 65°C, scintillant added and phosphorylated peptide quantified in a scintillation counter (Trilux, PerkinElmer)

HEPES is N-[2-Hydroxyethyl]piperazine-N'-[2-ethanesulfonic acid]
BSA is bovine serum albumin.

Claims.

1. The use of a compound of formula (I) or a salt, N-oxide, hydrate or solvate thereof, in the preparation of a composition for inhibition of CDK2 activity:



wherein

Rings A and B are each optionally substituted aryl or heteroaryl,

Alk represents an optionally substituted divalent C₁-C₆ alkylene radical;

n is 0 or 1;

Q represents a radical of formula -(Alk¹)_p-(X)_r-(Alk²)_s-Z wherein in any compatible combination

Z is hydrogen or an optionally substituted carbocyclic or heterocyclic ring,

Alk¹ and Alk² are optionally substituted divalent C₁-C₆ alkylene radicals,

X represents -O-, -S-, -(C=O)-, -(C=S)-, -SO₂-, -SO-, -C(=O)O-, -OC(=O)-, -C(=O)NR^A-, -NR^AC(=O)-, -C(=S)NR^A-, -NR^AC(=S)-, -SO₂NR^A-, -NR^ASO₂-, -OC(=O)NR^A-, -NR^AC(=O)O-, or -NR^A- wherein R^A is hydrogen or C₁-C₆ alkyl, and

p, r and s are independently 0 or 1.

2. The use as claimed in claim 1 wherein rings A and B are, independently phenyl, naphthyl, 2-,3- or 4-pyridyl, 5-pyrimidinyl, 2- or 3-thienyl, 2- or 3-furyl.
3. The use as claimed in claim 1 wherein each of rings A and B are phenyl.
4. The use as claimed in any of claims 1 to 3 wherein rings A and B are substituted by methyl, ethyl, methylenedioxy, ethylenedioxy, methoxy, ethoxy, methylthio, ethylthio, hydroxy, hydroxymethyl, hydroxyethyl, mercapto, mercaptomethyl, mercaptoethyl, amino, mono- or di-methylamino, mono- or di-ethylamino, fluoro, chloro, bromo, cyano, N-morpholino, N-piperidinyl, or N-piperazinyl, the latter being optionally C₁-C₆ alkyl- or benzyl-substituted on the free ring nitrogen.
5. The use as claimed in any of claims 1 to 4 wherein n is 0.
6. The use as claimed in any of claims 1 to 5 wherein n is 1 and Alk is -CH₂-, -CH₂CH₂-, -CH₂CH(CH₃)-, or CH₂CH₂CH₂-.
7. The use as claimed in any of the preceding claims wherein each of p, r and s is 0, and Z is hydrogen.
8. The use as claimed in any of claims 1 to 6 wherein p, r and s are each 0, and Z is an optionally substituted phenyl, cyclohexyl, pyridyl, morpholino, piperidinyl, or piperazyl ring..
9. The use as claimed in any of claims 1 to 6 wherein p and/or s is 1 and r is 0, such that Z is linked to ring A by an alkylene radical
10. The use as claimed in any of claims 1 to 6 wherein each of p, r, and s is 1, such that Z is linked to Ar¹ by an alkylene radical which is interrupted by the hetero atom-containing X radical.

11. The use as claimed in any of claims 1 to 6 wherein p and s are 0 and r is 1, such that Z is linked to Ar¹ via the hetero atom-containing X radical.
12. The use as claimed in any of claims 1 to 6 wherein p is 0, r is 1, and X is a sulfonamide radical -NR^ASO₂- or a carboxamide radical -NR^AC(=O)-, wherein R^A is hydrogen or a (C₁-C₆)alkyl group, with the N atom linked to the ring A.
13. The use as claimed in claim 12 wherein s is 1 and Z is hydrogen.
14. The use as claimed in claim 12 wherein s is 0 and Z is an optionally substituted carbocyclic or heterocyclic ring.
15. The use as claimed in claim 14 wherein Z is optionally substituted phenyl.
16. A method of treatment of diseases or conditions mediated by excessive or inappropriate CDK2 activity in mammals, particularly humans, which method comprises administering to the mammal an amount of a compound of formula (I) as defined in any of the preceding claims, or a salt, hydrate or solvate thereof, effective to inhibit said CDK2 activity.
17. A compound of formula (I) as defined in any of claims 1 to 15, or a salt hydrate or solvate thereof, for use in human or veterinary medicine, particularly in the treatment of diseases or conditions mediated by excessive or inappropriate CDK2 activity.
18. The use as claimed in any of claims 1 to 15, a method of treatment as claimed in claim 16, or a compound for use as claimed in claim 17 wherein the CDK activity is associated with cancer, psoriasis or restenosis.

PCT/GB2004/001214

